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ORIGINAL ARTICLE

Distribution of fatty acids in triacylglycerols from ungrafted *Desi Mango* (*Mangifera indica*) kernel lipids

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Abstract The lipids of ungrafted '*Desi*' mango (*Mangifera indica*) kernel were extracted with chloroform/methanol, purified and triacylglycerols (82.6%) were separated by thin layer chromatography (*TLC*). The pure triglycerides are fractionated into five bands by 25% silver nitrate-impregnated thin layer chromatography (*Ag-TLC*). The composition and position of fatty acids at α, α′ and β-positions of fractionated/unfractionated triacylglycerols were determined by the use of pancreatic lipase, sodium tetraborate-impregnated thin layer chromatography (*borate-TLC*) and gas liquid chromatography (*GLC*). The highest percentage of the most saturated band was 55.6% which comprised of *sn*-1,3-disaturated-2-oleoglycerols and *sn*-1(3)saturated-2,3(1,2)-dioleoylglycerols. The oleic, linoleic and linolenic acids occupied β-position depending upon comparatively higher percentage of unsaturated fatty acids in specific fraction.

1. Introduction

The mango belongs to genera *Mangifera indica* of *Anacardiaceae* family. There are 76 genera and 600 species of trees and shrubs mainly tropical in distribution but several species extending into southern Europe, temperate Asia and America (Janick and Paull, 2008). The mango seed kernels, waste material of juice and pulp producing factories, can be utilized for the production of oils/fats which may be used either for edible purposes or for the preparation of other industrial products. The effort has been made for thorough investigation on the distribution of fatty acids in triacylglycerols prior to the utilization of mango fat as such or after its fractionation. The *M. indica* kernel oil of ungrafted variety locally known as *Desi* contains 60.51% of total saturated fatty acids (*TSFA*) and 39.49% of total unsaturated fatty acids (*TUFA*). The percentage of stearic acid is 81.31% of *TSFA* and oleic acid is 78.75% of *TUFA* (Ali et al., 2007). The mango kernels 1,037,200 tonnes, can be utilized for the production of 4550 tonnes of fat per annum. The percentage of extracted fat on

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the basis of dried kernels is; 11.8% and this fat contain 82.6% of triacylglycerols (Ali et al., 2009).

However, the previous work did not report about the positional distribution of fatty acids in triacylglycerols. The triacylglycerols comprise the major portion of extracted lipids in vegetable oils/fats (Gunstone et al., 1986) and distribution of fatty acids in triglycerides plays an important role for the characterization of different vegetable oils/fats.

The different fractions of triglycerides depending upon the fatty acids and their attachments are necessary for the specific applications of vegetable oils/fats. The various methods are employed by different workers such as esterification (inter-, trans- and directed), hydrogenation (selective or homogeneous), fractionation (dry, with solvent or a water containing a surface active-agent) or combination of these in order to isolate a product with selective fatty acids, specific melting points, a sufficient degree of hardness and brittleness of the product at room temperature (Baliga and Shitole, 1981). The various fractionated products of Malaysian *Palm* oil are also commercially available i.e., *Palm olein*, *Palm sterin*, vegetable Ghee, shortening, ice cream fat, coating fat, margarine, dough fat, *Cocoa butter* substitute and cream fat (Bathich, 2009). These products have been fractionated on the basis of the structure of triglycerides and the attached fatty acids. Keeping in view of the importance of triacylglycerols for specific applications, the structural analysis of triacylglycerols has been carried out for the utilization of extracted fats from the waste kernels.

2. Experimental

2.1. Materials

Solvents and reagents used were of analytical-grade mostly purchased from Merck-Darmstadt, Germany and Riedel-de-Haën, Germany. Silica gel HF_{254}, Merck Ref. 7739 was used for *TLC* and most of the mono, di and triacylglycerols' standards are products of BDH, UK. The silver nitrate and borax of Winlab, UK were also used for the preparation of thin layer chromatograms. BF_3-methanol complex (Merck-Schuchardt, Germany) used for esterifications of fatty acids. The standards; methyl esters of fatty acids were attained from Supelco®, USA for *GLC* analysis. The non-destructive locating reagent 2,7-dichlorofluorescein (Merck, Germany) used for the colored spots of lipid compounds under ultra violet light; λ 366 nm. The pancreatic lipase (Calibiochem-Behring Corp, USA) was used for the selective hydrolysis of triacylglycerols.

2.2. Methodology

The structural analysis of triacylglycerols may be carried out by its fractionation with silver nitrate-impregnated thin layer chromatography (*Ag-TLC*) (Daneshrad, 1978). The composition of fatty acids as methyl esters of triacylglycerols and fractions is determined by gas liquid chromatography (*GLC*). The pancreatic lipase (Mattson and Volpenhein, 1961; Fiume and Viosti, 1989; Subbaram and Youngs, 1967; Conacher et al., 1970) is used for the selective hydrolysis (lipolysis) of triacylglycerols and fractions separately. The lipase hydrolysis of the triacylglycerols and its fractions is carried out on small quantities of triacylglycerols. In this method pancreatic lipase brings about the preferential hydrolysis of fatty acids in the terminal positions of triacylglycerols. The borax is used for the chromatographic separation of diacylglycerols and monoacylglycerols (Thomas et al., 1963). The fatty acids attached to these partial triacylglycerols at α, α′ and β-position are liberated, methylated and analyzed by gas chromatography (*GLC*) (Fig. 1).

2.3. Extraction of lipids

The mango seeds were washed, dried and crushed; the kernels were dried and ground. The solvent mixture of chloroform–methanol (2:1) 500 mL was used for the extraction of lipids from dried powder (50 g) by stirring with a magnetic stirrer at room temperature. The meal was filtered off under vacuum with washings of the residue. The process was repeated with half quantity of solvent for maximum extraction of lipids and extracts were pooled together in a round-bottomed flask. The solvent was removed under vacuum by rotary film evaporator (Hëidolph, Germany) and purified the lipids by treating with a mixture of chloroform–methanol–

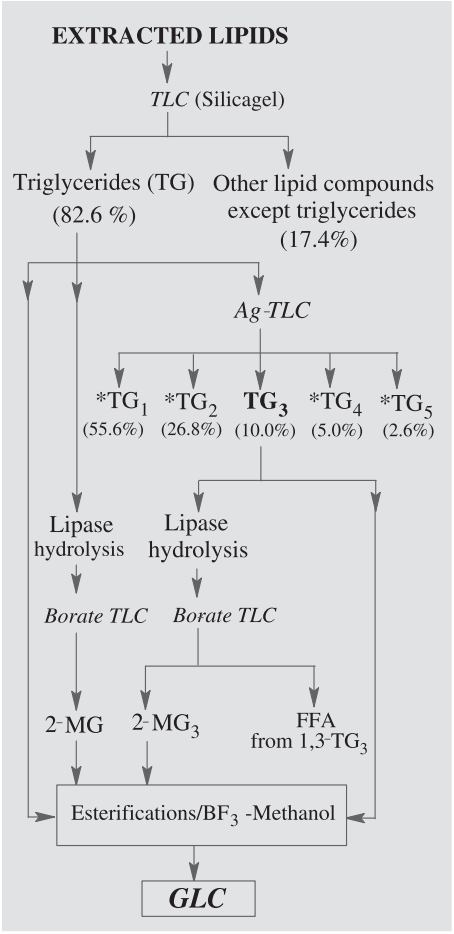

Figure 1 Schematic presentation for the positional analysis of fatty acids in triglycerides from ungrafted *Desi Mango* (*Mangifera indica*) kernel. *The processes for TG1, TG_2, TG_4 and TG_5 are same as for TG_3.

sodium chloride solution (0.9%): (3:48:47). The fatty matter (5.9 g) was stored under nitrogen for further work.

2.4. Separation of triacylglycerols

The separation of triacylglycerols from extracted lipids was carried out by *TLC*. Ten thin layer chromatograms (20 × 20 cm) of thickness 0.5 mm were prepared by the use of silicagel; HF_{254}, Merck Ref. 7739 (Stahl, 1966). The lipid (400 mg) gave triacylglycerols (330.4 mg) having R_f value; 0.61. The developing solvent mixture; *n*-hexane-diethyl ether (90:10) was used for the separation of triacylglycerols.

2.5. Fractionation of triacylglycerols

Five $AgNO_3$-impregnated thin layer chromatograms (20 × 20 cm) of 0.5 mm thickness were prepared by using silicagel; HF_{254}, Merck Ref. 7739 and silver nitrate (Barrett et al., 1968) for the separation of triacylglycerols (200 mg) into five fractions. The developing solvent mixture benzene–diethyl ether (9:1) was used for elution. The non-destructive locating reagent 2,7-dichloroflourescein was used to locate pink colored bands under UV light (Universal UV Lampe, Cammag, Schweiz) at λ 366 nm. The fractions were eluted with chloroform and the solvent was removed by rotary film evaporator to find out the five different fractions i.e., first fraction (111.2 mg), second fraction (53.4 mg), third fraction (20.0 mg), fourth fraction (10.0 mg) and fifth fraction (5.2 mg).

2.6. Pancreatic hydrolysis of triacylglycerols and fractions

The pancreatic hydrolysis of fractionated and unfractionated triacylglycerols was carried out under the recommended methods of previous workers. A glass tube (20 mL) with a stopper containing either 20 mg unfractionated or fractionated triacylglycerols, the solvent di-isopropyl ether (0.5 μL), water (5 μL) and pancreatic lipase 20 μg was shaken for one hour in shaking water bath at 50 °C (Akhtar et al., 1975; Luddy et al., 1964). The glass tube was cooled to room temperature and the contents were diluted with the addition of 0.5 mL solvent. The mixture was centrifuged at 2000 rpm for the separation of material into two layers, the upper layer was removed and the hydrolysed product was obtained after the removal of solvent.

2.7. Separation of fatty acids liberated at α, α′-position and 2-monoacylglycerols

The fatty acids liberated at α, α′(1,3)-position and β(2-monoacylglycerols)-position produced as a result of pancreatic lipase hydrolysis of unfractionated/fractionated triacylglycerols were separated by the application of sodium tetraborate-impregnated *TLC* (Thomas et al., 1963). The silicagel 60 g and distilled water 240 mL were used for the preparation of ten chromatograms (20 × 20 cm) of 0.5 mm thickness. The chromatograms were activated at 105 °C for an hour. The hydrolysed material (30 mg) was separated by using solvent system; benzene–diethylether–ethanol–glacial acetic acid (50: 40:2:0.2) (Freeman and West, 1966).

2.8. Infrared spectroscopy and gas chromatography

The *FTIR* spectroscope (Thermo-Nicolet IR-200) was used for the conformation of purified triglycerides, and showed absorptions at 1370 cm−1 (CH_3 bend), 1460 cm^{-1}, (CH_2 bend), 1740 cm^{-1} (C═O stretch), 2840 cm^{-1} (CH_3 stretch) and 2930 cm^{-1} (CH_2 stretch). The gas liquid chromatograph; *GC-14A* and data processor *C-R-4A* were used for the identification of methyl esters by using a polar column (2.5 m × 3 mm id) with coating material GP-10%-SP-2330 on supporting media 100–120 chromosorb WAW. The hydrogen flame ionization detector (FID) was used with requisite temperature of detector and injector at 250 and 230 °C, respectively. It was operated under temperature programming 180–210 °C at the rate of 4 °C/min and Nitrogen flow rate of 30 mL/min. The fatty acid methyl esters were identified by the comparison of their corresponding retention times with standard methyl esters of fatty acids; C_{12}–C_{24} (Supelco®, USA) under the same conditions.

3. Results and discussion

The triacylglycerols separated from the mango kernel lipids of ungrafted *Desi* variety by *TLC* having R_f value (0.61) as shown in Fig. 2 was fractionated into five bands by *Ag-TLC* (Fig. 3). The fractionation of pure triacylglycerols is accomplished according to the degree of unsaturation of attached fatty acids. The percentage of upper most fraction-1 (55.6%) being most saturated is highest in as compared to others i.e., fraction-2 (26.8%), fraction-3 (10.0%), fraction-4 (5.0%) and fraction-5 (2.6%). The fatty acid compositions as methyl esters, prepared by treating triacylglycerols/fractionated triacylglycerols with BF_3-methanol reagent (AOCS, 1997) are determined as shown in Table 1.

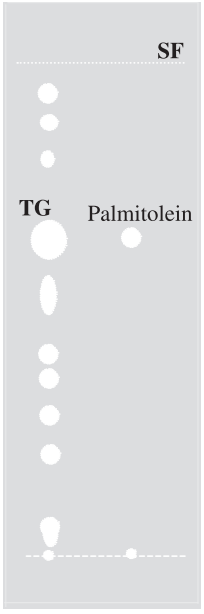

Figure 2 *TLC* of extracted lipids for the separation of triacylglycerols. Solvent system; *n*-hexane-diethylether-acetic acid (80:20:2) R_f value of triacylglycerols (TG); 0.61.

Figure 3 Fractionation of purified triglycerides by *TLC* solvent system; benzene-diethyl ether (90:10).

Table 1 Fatty acids composition (%) of unfractionated and fractionated triacylglycerols of *Desi Mango* (*Mangifera indica*) kernel.

Fatty acids	Triacylglycerols					
	TG[a]	TG_1	TG_2	TG_3	TG_4	TG_5
$C_{12:0}$	0.2	0.1	0.3	0.1	–	–
$C_{14:0}$	0.8	0.9	0.8	0.6	0.4	0.1
$C_{16:0}$	8.4	10.2	7.4	6.8	5.2	3.8
$C_{16:1}$	0.2	0.4	0.6	0.9	0.6	0.3
$C_{18:0}$	47.4	49.2	40.6	35.9	30.5	26.6
$C_{18:1}$	35.5	34.2	46.4	50.7	48.4	45.1
$C_{18:2}$	2.7	–	–	1.6	4.6	10.8
$C_{18:3}$	2.2	–	–	–	7.6	12.4
$C_{20:0}$	2.1	3.4	2.1	1.4	0.9	0.6
$C_{20:1}$	0.5	1.6	1.8	2.0	1.8	0.3

[a] TG represents unfractionated triacylglycerols while TG_1, TG_2, TG_3, TG_4 and TG_5 are the fractions of triacylglycerols.

The combination of *TLC* and *GLC* studies shows that the percentage of *TSFA* (63.8%) is highest in fraction-1 with major percentage of stearic acid (49.2%), whereas the percentage of *TUSFA* fatty acids (36.2%) is lowest in this fraction. The oleic acid (35.5%) is the prominent unsaturated fatty acid and *PUFA* have not been found out in fraction-1. The percentages of *TSFA* and *TUSFA* in fractions; 2, 3 and 4 are 51.2/48.8%, 44.8/55.2% and 37.0/63.0%, respectively. The fractions; 2, 3, 4 and 5 contain the higher percentages of oleic acids i.e., 46.4%, 50.7%, 48.4%, and 45.1% respectively in comparison to fraction-1. The fraction-5 contains the lowest percentage of *TSFA* (31.1%) and highest percentage of *TUFA* (68.9%).

Table 1 shows that the extent of saturation of triacylglycerols is in decreasing order from fraction-1 to fraction-5, whereas triacylglycerols with monounsaturated fatty acids increase from fraction-1 to fraction-3, but decrease from fraction-4 to fraction-5. The diunsaturated triacylglycerols show their presence in fraction-3 to fraction-5 in increasing order. The fraction-4 and fraction-5 show the presence of triunsaturated triacylglycerols in increasing order. The fraction-1 is being highly saturated and is considered comparatively nearest to the solvent front. The positions of fatty acids at α, α′-position and β-position of triacylglycerols are found out by using pancreatic lipase hydrolysis which liberate fatty acids at α, α′-position without disturbing β-position of triacylglycerols. The liberated fatty acids, 2-monoacylglycerols and unreacted triacylglycerols are separated by 4.3% sodium tetraborate-impregnated thin layer chromatography (*borate-TLC*). These are converted into methyl esters and analyzed by *GLC*.

The fatty acid compositions of 2-monoacylglycerols (2-MG) of total triacylglycerols (TG) and fractionated triacylglycerols are also determined by *Ag-TLC* as shown in Table 2. The results show the highest percentage of oleic acid (72.9%) in β-monoacylglycerol from unfractionated triacylglycerols which was separated by *borate-TLC* whereas, the percentage of oleic acid is 49.4% in fraction-1 and the percentage of oleic acid is steadily increasing to fraction-5 i.e., 70.3%. The stearic acid (12.5%) constitutes the second

Table 2 Fatty acids composition (%) of 2-monoacylglycerols from unfractionated and fractionated triacylglycerols of *Desi* variety.

Fatty acids	2-Monoacylglycerols					
	2-MG[a]	$2\text{-}MG_1$	$2\text{-}MG_2$	$2\text{-}MG_3$	$2\text{-}MG_4$	$2\text{-}MG_5$
$C_{12:0}$	0.3	0.2	0.1	Traces	–	–
$C_{14:0}$	0.7	0.5	0.4	0.2	0.1	Traces
$C_{16:0}$	4.3	4.0	3.8	2.8	1.7	1.0
$C_{16:1}$	0.6	0.4	0.6	1.2	1.9	2.1
$C_{18:0}$	12.5	42.2	35.5	23.7	13.2	5.0
$C_{18:1}$	72.9	49.4	56.3	63.1	66.5	70.3
$C_{18:2}$	3.1	–	–	6.3	8.5	10.4
$C_{18:3}$	2.3	–	–	–	5.6	8.6
$C_{20:0}$	2.6	2.3	2.0	0.9	0.5	0.2
$C_{20:1}$	0.7	1.0	1.3	1.8	2.0	2.4

[a] 2-MG represents unfractionated 2-monoacylglycerols from TG, while $2\text{-}MG_1$, $2\text{-}MG_2$, $2\text{-}MG_3$, $2\text{-}MG_4$ and $2\text{-}MG_5$ are the fractions of TG_1, TG2, TG_3, TG_4 and TG_5, respectively.

Table 3 The range of fatty acids' composition (mole%) of fractionated triacylglycerols and 2-monoacylglycerols of *Desi* variety.

Fatty acids	Fractionated triacylglycerols	Fractionated 2-monoacylglycerols
$C_{12:0}$	0–0.3	0–0.2
$C_{14:0}$	0.1–0.9	Traces–0.5
$C_{16:0}$	3.8–10.2	1.0–4.0
$C_{16:1}$	0.3–0.9	0.4–2.1
$C_{18:0}$	26.6–49.2	5.0–42.2
$C_{18:1}$	34.2–50.7	49.4–70.3
$C_{18:2}$	1.6–10.8	6.3–10.4
$C_{18:3}$	7.6–12.4	5.6–8.6
$C_{20:0}$	0.6–3.4	0.2–2.3
$C_{20:1}$	0.3–2.0	1.0–2.4

highest percentage after oleic acid in β-monoacylglycerols from unfractionated triacylglycerols and is in decreasing order from fraction-1 to fraction- 5. The *PUFA* i.e., linoleic and linolenic acids are absent in fraction-1, fraction-2 and the percentage of *PUFA* of 2-monoacylglycerols is continuously increasing from fraction-3 to fraction-5. The highest percentages of these acids have been found out in fraction-4 and fraction-5. The range of fatty acids composition of fractionated triacylglycerols by *Ag-TLC* and also the fatty acids' percentile range liberated from 2-monoacylglycerols procured after hydrolysis by pancreatic lipase of five fractions are given in Table 3. However, the *Ag-TLC* proves the distribution of fatty acids as regards to saturation, monoenoic, dienoic and trienoic fatty acids. Therefore, the fatty acids may be abbreviated as; *S*, *O*, *L* and L_e (Fig. 4) for saturated, monoenoic, dienoic and trienoic, respectively.

Fraction-1	:	*SSO\ SOS*
Fraction-2	:	*SOO\ OSO*
Fraction-3	:	*OOO\ SOL\ SLO*
Fraction-4	:	*OLL\ LOL\ LLeO\ LeLO*
Fraction-5	:	*OLeLe\ LeOLe\ LLeLe\ LeLLe*

Figure 4 The prominent triacylglycerols of ungrafted *Desi Mango* kernel fat. Where *S*: $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20:0}$; *O*: $C_{16:1}$, $C_{18:1}$, $C_{20:1}$; *L*: $C_{18:2}$; L_e: $C_{18:3}$.

The triacylglycerols from mango kernel lipids have been deduced on the basis of *Ag-TLC*, analysis of fatty acids in different triacylglycerols' fractions and fatty acids of their corresponding 2-monoacylglycerols after lipolysis and *borate-TLC*. The triacylglycerols composition obtained by 25% *Ag-TLC* (Bezard and Bugaut, 1972; Brockerhoff, 1975) is in agreement with 1,3-random, 2-random distribution hypothesis (Ikramulhaq and Ehteshamuddin, 1971; Char et al., 1977) and the earlier work (Sergiel, 1973; Ahmed and Raie, 1992; Rehman and Chaudhari, 2005).

The highest percentage of oleic acid has been determined at *sn*-2 position of triacylglycerols which can be replaced by linoleic and linolenic acid depending upon comparatively high percentage of unsaturation in a specific fraction. The previous literature also reveals the distribution of oleic acid, linoleic acid and linolenic acid at β-position (Mattson and Volpenhein, 1963; Gunstone et al., 1965). The pancreatic lipase hydrolysis of triacylglycerols and fractionated triacylglycerols also supports the above mentioned hypothesis (Jurriens et al., 1964; Wal, 1964; Sangupta and Choudhary, 1978). The structures of triacylglycerols of fraction-1 to fraction-5 separated by *Ag-TLC* are shown in Fig. 5. The β-position of triacylglycerols occupied by unsaturated fatty acids can be named as *sn*-2-oleo-1,3-distearin, *sn*-2-oleo-1-stearin-3-olein, triolein, *sn*-2-oleo-1-stearin-3-linolein, *sn*-2-oleo-1,3-dilinolein, *sn*-2-linoleo-1-stearin-3-olein, *sn*-2-linoleo-1-olein-3-linolein, *sn*-2-lioleo-1-linolenin-3-olein, *sn*-2-linoleo-1,3-dilinolenin, *sn*-2-linolineo-1-linolein-3-olein, *sn*-2-linolineo-1-olein-3-linolenin,

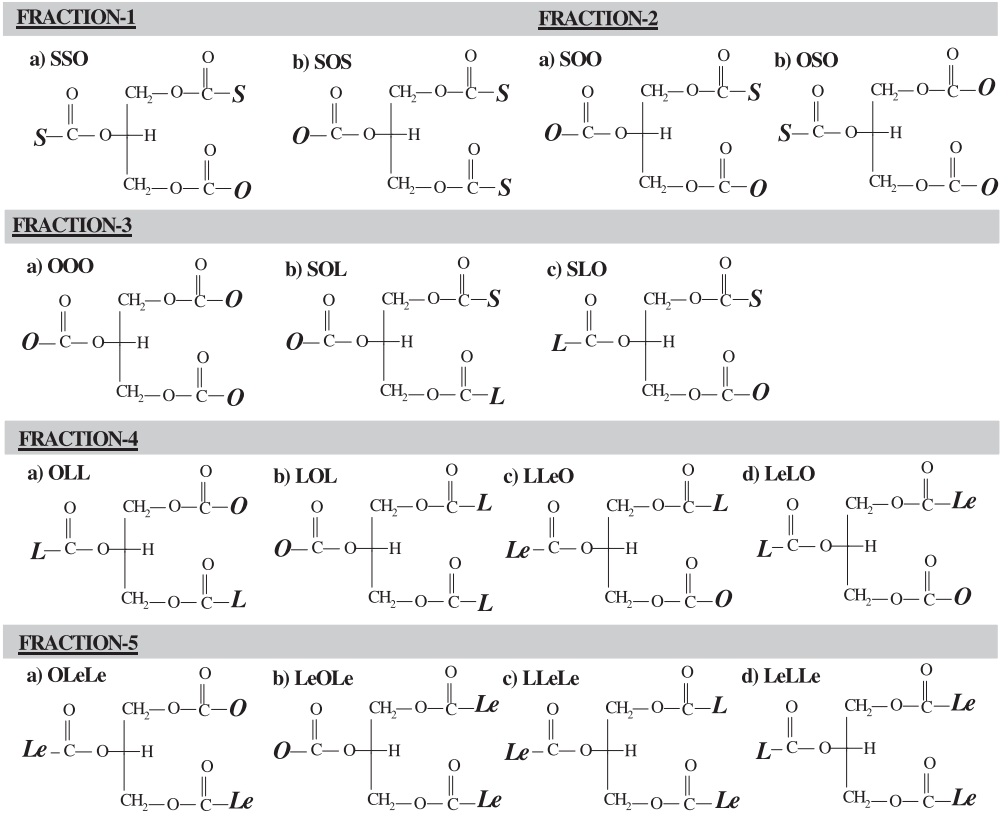

Figure 5 The structures of triacylglycerols from *Desi Mango* (*Mangifera indica*) kernel lipids in different fractions by *Ag-TLC*. The abbreviations; *S*, *O*, *L* and L_e are used for saturated, monoenoic, dienoic and trienoic fatty acids.

sn-2-linoleneo-1-linolein-3-liolenin. The prominent triacylglycerols of mango kernel; *sn*-1,3-disaturated-2-oleoglycerols of fraction-1 and *sn*-1(3)saturated-2,3(1,2)-dioleoylglycerols of fraction-2 are comparable with *Cocoa butter* and *Shea* butter therefore, it can be used for the preparation of confectionary and chocolates formulations (Hilditch and Williams, 1964; Jurriens, 1968).

4. Conclusion

The fractionated triacylglycerols isolated from the lipids of waste *M. indica* kernels of *Desi* variety indicates that the uppermost fraction being highly saturated is in highest percentage. It is also concluded that highest percentage of oleic acid has been determined at *sn*-2 position of triacylglycerols which can be replaced by linoleic and linolenic acid depending upon comparatively high percentage of unsaturation in a specific fraction. The major triacylglycerols of mango kernel consists of *sn*-1,3-disaturated-2-oleoglycerols (SOS) and *sn*-1(3)saturated-2,3(1,2)-dioleoylglycerols (SOO/OOS). Such compositions are comparable with *Cocoa* and *Shea* butter. So, the kernels' semisolid fat as it is can be used for the preparation of confectionary and chocolates formulations. The mango kernel fat can also be fractionated for specific applications without hydrogenation/esterification like the utilization of Malaysian *Palm* oil fractions.

References

Ahmed, I., Raie, M.Y., 1992. Proc. Pak. Acad. Sci. 29 (3), 203–211.

Akhtar, M.W., Blain, J.A., Patterson, J.D.E., 1975. Pak. J. Sci. Res. 27 (1–4), 212–216.

Ali, Z., Siddiqui, H.L., Hamid, S., 2007. Sci. Int. (Lahore) 19 (1), 51–54.

Ali, Z., Siddiqui, H.L., Hamid, S., 2009. J. Chem. Soc. Pak. 31 (1), 131–137.

American Oil Chemist's Society, 1997. Official and Recommended Practices of the AOCS, fifth ed. AOCS Press, Champaign, pp. 1b–89.

Baliga, B.P., Shitole, A.D., 1981. J. Am. Oil Chem. Soc. 58 (2), 110–114.

Barrett, C.B., Dallas, M.S.J., Padley, P.B., 1968. J. Am. Oil Chem. Soc. 40 (10), 580–584.

Bathich, Y., 2009. Palm Oil Products Specifications. Market Square, Selangor, D.E, Malaysia. Available from: <http://market-square-malysia.com>.

Bezard, J., Bugaut, M., 1972. J. Chromatogr. Sci. 10, 451.

Brockerhoff, H., 1975. In: Lowenstein, J.M. (Ed.), . In: Methods in Enzymology, vol. 35B. Academic Press, New York, pp. 315–325.

Char, B.L. Narsimha, Reddy, B.R., Thirumala Rao, S.D., 1977. J. Am. Oil Chem. Soc. 54 (11), 494–495.

Conacher, H.B.S., Gunstone, F.D., Hornby, G.M., Padley, F.B., 1970. Lipids 4, 434–441.

Daneshrad, A., 1978. J. Am. Oil Chem. Soc. 55 (3), 317–319.

Fiume, F., Viosti, D., 1989. Riv. Ital. Sostanze Grasse 65 (6), 445–446.

Freeman, C.P., West, D.J., 1966. J. Lipid Res. 1, 324–327.

Gunstone, F.D., Hamilton, R.J., Padley, F.B., Qureshi, M.I., 1965. J. Am. Oil Chem. Soc. 42 (11), 965–970.

Gunstone, F.D., Harwood, J.L., Padley, F.B., 1986. The Lipid Hand Book, vol. 1. Chapman and Hall, New York, pp. 51–54.

Hilditch, T.P., Williams, P.N., 1964. The Chemical Composition of Natural Fats, vol. 4. Chapman and Hall, London, pp. 431–444.

Ikramulhaq, M.Y., Ehteshamuddin, F.M., 1971. Sci. Ind. 8, 207.

Janick, J., Paull, R.E., 2008. The Encyclopedia Fruits & Nuts. Cambridge University Press, Cambridge, pp. 30.

Jurriens, J., De-Vries, B., Schouten, L., 1964. J. Lipid Res. 5, 366–368.

Jurriens, J., 1968. In: Boekenoogen, H.A. (Ed.), Analysis and Characterization of Oils, Fats and Fat Products, vol. 2. Interscience Publishers, London, pp. 217–298.

Luddy, F.E., Barford, R.A., Herb, S.F., Magidman, P., Riemenschneider, R., 1964. J. Am. Oil Chem. Soc. 41 (10), 693–696.

Mattson, F.H., Volpenhein, R.A., 1961. J. Biol. Chem. 236 (7), 1891–1894.

Mattson, F.H., Volpenhein, R.A., 1963. J. Lipid Res. 4, 392–396.

Rehman, S., Chaudhari, T.A., 2005. Pak. J. Sci. Ind. Res. 48 (6), 389–392.

Sangupta, A., Choudhary, S.K. Ray, 1978. J. Am. Oil Chem. Soc. 55 (8), 621–624.

Sergiel, J.P., 1973. Rev. Fr. Crops Gras. 3, 137.

Stahl, E., 1966. Pharmazie 11, 633.

Subbaram, M.R., Youngs, C.G., 1967. J. Am. Oil Chem. Soc. 44 (7), 425–428.

Thomas, A.E., Scharoon, J.E., Ralston, H., 1963. J. Am. Oil Chem. Soc. 42 (9), 789–792.

Wal, R.J. Vander, 1964. In: Paoletti, P., Kritchevsky, D. (Eds.), . In: Advances in Lipid Research, vol. 2. Academic press, New York, pp. 1–16.